Patent
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Knapp et al.

Parent Application No.: 09/122,477

Parent Filed: July 23, 1998

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For: PHOTON REDUCING AGENTS FOR FLUORESCENCE ASSAYS

Group Art Unit: Unassigned

Examiner: Unassigned

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Mikhail Bayley

Commissioner for Patents
Washington D.C., 20231

Sir:

PRELIMINARY AMENDMENT

Prior to the examination of the present application, please amend the application as follows:

AMENDMENTS

IN THE SPECIFICATION

On page 1, under the heading "**PHOTON REDUCING AGENTS FOR FLUORESCENT ASSAYS**", please delete the sentence,

"This application claims the benefit of priority under 35 U.S.C. §119(e) to United States provisional application No. 60/054,519, filed 8/1/97."

In its place please insert the text below.

--This is a continuation of United States patent serial number 09/122,477 filed July 23, 1998, now allowed, which claims the benefit of U.S. Provisional Application No. 60/054,519 filed August 1st 1997.--

IN THE CLAIMS

Please cancel claims 1 to 73 and 78 to 79 and add claims 80 to 116 as below.

--80. A system for fluorescence assays, comprising:

a) a source of excitation light, for fluorescent excitation;

b) a detector, for measuring emission; and

c) an aqueous sample, comprising;

i) a plurality of living cells in contact with a solid surface,

ii) a first reagent, comprising a photon producing agent,

iii) a second reagent comprising a photon reducing agent, wherein said photon reducing agent is substantially impermeant to a plasma membrane of a living mammalian cell,

wherein said photon reducing agent does not specifically bind to said membrane compartment,

wherein said photon reducing agent has an absorption spectrum that overlaps with the absorption, emission or excitation spectrum of said photon producing agent, and

wherein said photon reducing agent is present in said aqueous sample at an amount sufficient to reduce light emitted from said aqueous sample by at least 10 % compared to the light emitted from said aqueous sample in the absence of said photon reducing agent.

81. The system of claim 80, wherein said solid surface is a bottom of a multiwell plate.

82. The system of claim 81, wherein said multiwell plate has between 6 and 3,456 wells.

83. The system of claim 81, wherein said multiwell plate has greater than 384 wells.

84. The system of claim 80, further comprising a temperature controller.

85. The system of claim 80, further comprising a multi-axis translation stage to move a multiwell plate.

86. The system of claim 80, further comprising auto focusing optics.

87. The system of claim 80, further comprising programmable control of imaging and data collection.

88. The system of claim 80, wherein said plurality of living cells comprises a target receptor.

89. The system of claim 80, wherein said plurality of living cells comprises a target ion channel.

90. The system of claim 80, wherein said plurality of living cells comprises a target intracellular nuclear receptor.

91. The system of claim 80, wherein said photon reducing agent is present in said aqueous sample at an amount sufficient to reduce light emitted from said aqueous sample by at least 30 % compared to the light emitted from said aqueous sample in the absence of said photon reducing agent.

92. The system of claim 80, wherein said photon reducing agent is present in said aqueous solution at an amount sufficient to reduce light emitted from said aqueous sample by at least 50 % compared to the light emitted from said aqueous sample in the absence of said photon reducing agent.

93. The system of claim 80, wherein said photon reducing agent is present in said aqueous sample at an amount sufficient to reduce light emitted from said aqueous sample by between 70 and 99 % compared to the light emitted from said aqueous sample in the absence of said photon reducing agent.

94. The system of claim 80, wherein said photon producing agent is selected from the group consisting of a fluorescent enzymatic substrate, a fluorogenic enzymatic substrate, a member of a FRET pair, a molecule that detects voltage across a membrane of a membrane compartment and an intracellular ion indicator.

95. The system of claim 94, wherein said photon producing agent is a fluorescent enzymatic substrate.

96. The system of claim 94, wherein said photon producing agent is a fluorogenic enzymatic substrate.

97. The system of claim 94, wherein said photon producing agent is a member of a FRET pair.

98. The system of claim 94, wherein said photon producing agent is a molecule that detects voltage across a membrane.

99. The system of claim 94, wherein said photon producing agent is an intracellular ion indicator.

100. The system of claim 80, wherein said photon reducing agent is selected from the group consisting of a collisional quencher, a particulate, an absorption quencher, a FRET quencher and a dark complex.

101. The system of claim 100, wherein said photon reducing agent comprises a particulate or colloidal quencher.

102. The system of claim 80, wherein said photon reducing agent comprises a light absorbing dye.

103. The system of claim 102, wherein said photon reducing dye is not a pH indicator dye.

104. The system of claim 100, wherein said photon reducing agent comprises a FRET quencher.

105. The system of claim 80, wherein said second reagent comprises at least two photon reducing agents.

106. The system of claim 105, wherein said second reagent comprises Tartrazine.

107. The system of claim 105, wherein said second reagent comprises chromotrope 2R.

108. The system of claim 105, wherein said second reagent comprises Acid Fuchsin.

109. The system of claim 105, wherein said second reagent comprises Patent Blue.

110. The system of claim 105, wherein said second reagent comprises Acid Red 37.

111. The system of claim 105, wherein said second reagent comprises chromotrope F8.

112. The system of claim 105, wherein said second reagent comprises Tartrazine.

113. The system of claim 80, wherein said photon reducing agent improves the optical signal to noise ratio by at least 300 % compared to the optical signal to noise ratio in the absence of said at least one photon reducing agent.

114. The system of claim 80, wherein the steady state concentration of said photon reducing agent within said plurality of living cells is less than 50 % of the concentration of said photon reducing agent outside of said plurality of living cells.

115. The system of claim 80, wherein the steady state concentration of said photon reducing agent within said plurality of living cells is less than 30 % of the concentration of said photon reducing agent outside of said plurality of living cells.

116. The system of claim 80, wherein the steady state concentration of said photon reducing agent within said plurality of living cells is less than 10 % of the concentration of said photon reducing agent outside of said plurality of living cells.--

REMARKS

Amendments to the specification

The amendments to the specification correct the priority information into the present specification. The amendment does not introduce new matter.

Amendments to the claims.

Applicants have canceled claims 1 to 73 and 78 to 79, and added new claims 80 to 116. These new claims do not introduce new matter as they are fully supported by the specification as originally filed. Support for specific claims includes that summarized in the table below.

Claim Number	Support
80	Page 13, line 25, page 20, lines 9 to 12, page 21, lines 7 to 16, page 22, lines 1 to 6, page 18, lines 2 to 6, page 20, lines 21 to 25, claim 49, claim 52 and claim 66.
81	Page 21, lines 9 to 10, claim 64
82	Page 21, lines 10 to 16, page 28, lines 3 to 5
83	Page 21, lines 10 to 16, page 28, lines 3 to 5
84	Page 29, lines 12 to 13, claim 65
85	Page 29, lines 13 to 15
86	Page 29, line 16
87	Page 29,lines 15 to 19
88	Page 23, lines 21 to 22
89	Page 23, lines 8 to 20
90	Page 26, lines 7 to 10
91	Page 18, lines 2 to 6
92	Page 18, lines 2 to 6
93	Page 18, lines 2 to 6
94	Page 15, lines 10 to 20
95	Page 15, lines 10 to 20

96	Page 15, lines 10 to 20
97	Page 15, lines 10 to 20
98	Page 15, lines 10 to 20
99	Page 15, lines 10 to 20
100	Page 15, line 29, and page 16 lines 1 to 17 claim 10
101	Page 16, lines 6 to 9
102	Claim 11
103	Claim 29
104	Page 15, line 29, and page 16, lines 1 to 2
105	Page 16, lines 25 to 29
106	Page 54, lines 13 to 16
107	Page 54, lines 13 to 16
108	Page 54, lines 13 to 16
109	Page 54, lines 13 to 16
110	Page 54, lines 13 to 16
111	Page 54, lines 13 to 16
112	Page 54, lines 13 to 16
113	Page 18, lines 23 to 25 and claim 40
114	Page 18, lines 2 to 6
115	Page 18, lines 2 to 6
116	Page 18, lines 2 to 6

In view of the foregoing, Applicants respectfully submit that the claims are in condition for allowance. Please apply any charges not covered, or any credits, to Deposit Account 50-1355.

Respectfully submitted,

Date 1/12/01

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